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(54) Title: METHOD TO TREAT RHEUMATOID ARTHRITIS

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HLA Dw4	K	D	L	L	E	Q	K	R	A	A	V	D	T	Y	C
HLA Dw2 a	K	D	F	L	E	D	R	R	A	A	V	D	T	Y	C
HLA Dw2 b	K	D	I	L	E	Q	A	R	A	A	V	D	T	Y	C
HLA Dw4 p	K	D	L	L	E	Q	K	R	A	A	V	D	T	Y	C
HLA Dw14 p	K	D	L	L	F	Q	R	R	A	A	V	D	T	Y	C
HLA Dw10 p	K	D	I	L	E	D	E	R	A	A	V	D	T	Y	C

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(57) Abstract

Subjects may be immunized against rheumatoid arthritis by administering the peptide Q(K/R)RAA in immunogenic form in a protocol leading to preferential expansion of suppressor and/or cytotoxic T-cells rather than helper T-cells. This preferential expansion can be effected by administration of interleukin-6 and/or cyclosporin A. Alternatively, the Q(K/R)RAA peptide coupled to the invariant region of a Class I histocompatibility sequence may be administered. Also disclosed are pharmaceutical compositions useful in the vaccination protocols.

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METHOD TO TREAT RHEUMATOID ARTHRITIS

Technical Field

The invention relates to regulation of the immune system to prevent and treat autoimmune diseases. More specifically, it concerns procedures to prevent and treat the immune response which is characteristic of rheumatoid arthritis in humans.

Background Art

The B-lymphocytes and macrophages from more than 90% of patients with seropositive rheumatoid arthritis express the Class II HLA protein DR4 or DR1, as defined serologically. It has been shown that DR4 specificity is present on several different haplotypes, three of which, Dw4, Dw14 and Dw15, are associated with the B-lymphocytes and macrophages from rheumatoid arthritis patients, and two of which, Dw10 and Dw13, are not. Thus, the B-lymphocytes and macrophages of subjects with rheumatoid arthritis express the Class II haplotypes Dw4, Dw14, Dw15 or DR1. It has previously been shown that the haplotypes associated with rheumatoid arthritis (RA) are distinguished from those not associated with RA by the sequence of amino acids 70-74 in the third hypervariable region of the DR-beta-1 chain (Gregersen, P., et al., Proc Natl Acad Sci USA (1986) 83:2642-2646; Gregersen, P., et al., Arch Rheum (1987) 30:1205-1213; Tonnelle, C., et al., Ann Inst Pasteur Immunol (1988) 139:41-53). The sequence of the region is either QKRAA (Dw4) or QRRAA (Dw14, Dw15

and DR1). The substitution of R (arginine) for K (lysine) is conservative. Thus, the sequence Q(K/R)RAA may be important in the development and symptoms of rheumatoid arthritis.

The current thinking with regard to the role of the proteins encoded by the major histocompatibility complex (MHC) in permitting the immune system to distinguish between self and nonself postulates that foreign antigens are "presented" to the various classes of T-cells by Class II proteins encoded by the MHC. The Class II protein contains a site capable of binding the foreign antigen (or a fragment thereof) and another site which recognizes a T-cell receptor. Structural studies by others have indicated that the position on the Class II MHC protein on which the Q(K/R)RAA sequence is located corresponds to the T-cell receptor recognition site.

Rheumatoid arthritis is thought to be an autoimmune disease wherein certain protein components of the synovial fluid are recognized as foreign by the immune system. Presumably they are presented to the T-lymphocytes in the context of an MHC Class II protein, which MHC Class II carrier is recognized as self by the T-lymphocyte. Based on the foregoing paragraphs, it may be supposed that this self-recognition site includes Q(K/R)RAA. It has now been found that by administering a suitable vaccine, the response of T-lymphocytes to the synovial fluid-associated antigen can be either diverted from the helper T-cell class, or prevented. The vaccines of the invention thus prevent T-helper proliferation in response to the synovial antigen conjugated to the Class II recognition site Q(K/R)RAA.

Disclosure of the Invention

The invention provides vaccines for the prevention and treatment of the autoimmune response to synovial protein which manifests itself in the symptoms of

rheumatoid arthritis. The vaccines of the invention effectively prevent the T-helper cell response to these proteins presented in the context of the MHC Class II histocompatibility protein having the sequence Q(K/R)RAA at its recognition site.

Thus, in one aspect, the invention is directed to a method to immunize a subject to prevent or ameliorate rheumatoid arthritis wherein the vaccination protocol comprises administration of an effective amount of the peptide Q(K/R)RAA in immunogenic form in conjunction with an effective amount of one or more immunostimulatory or immunosuppresant substances which preferentially enhance the proliferation of suppressor and/or cytotoxic T-cells, and/or suppress the expansion of helper T-cells without effect on suppressor/cytotoxic expansion. Such immunoaffecting substances include interleukin-6 (IL-6), cyclosporin A, antibodies against CD4 antigen, transforming growth factor-beta (TGF-beta), and combinations thereof. The invention is also directed to vaccines useful in this method.

In another aspect, the invention is directed to a vaccine useful in preventing rheumatoid arthritis, which vaccine comprises the Q(K/R)RAA amino acid sequence coupled to an invariant portion of a Class I histocompatibility sequence, and to methods of rheumatoid arthritis prevention and amelioration using this vaccine.

Brief Description of the Drawings

Figure 1 shows the stimulation (or not) of T-cells derived from EBV-exposed and EBV-nonexposed subjects by gp110 and by Dw4 peptide.

Figure 2 shows antibody titers measured against gp110 peptide or protein of a rabbit immunized with gp110 peptide.

Figure 3 shows immunoblots of HLA Class II Dw4 and Dw2 proteins with rabbit anti-DW4 antisera.

Figure 4 shows the comparative amino acid sequences of Dw4, Dw14 and Dw10 beta peptides.

Modes of Carrying Out the Invention

The vaccines of the invention utilize as an essential component the amino acid sequence Q(K/R)RAA in an immunogenic form or an immunogenic form of the peptide sequence which forms that portion of a T-cell receptor binding to this epitope. Accordingly, the specific peptide hapten sequence may be part of a larger polypeptide or protein and/or may be conjugated, optionally through a linker, to a carrier. If the peptides are included within a larger protein, convenient larger proteins include additional amino acid residues found in the Class II DR-beta-1 chain or additional amino acid residues adjacent this peptide in glycoprotein gp110 associated with the Epstein-Barr virus; for peptides representing the Q(K/R)RAA sequence in the context of the Class I MHC protein constant regions, extensions of these regions may also be employed. However, the individual peptide may also be used. In both cases arbitrary, non-interfering chain extensions may also be employed.

Suitable carriers which enhance immunogenicity of the hapten, or confer immunogenicity upon it, include the commonly employed keyhole limpet hemocyanin (KLH) protein, tetanus toxoid, or other antigenically neutral materials, such as human serum albumin. If a separate carrier such as tetanus toxoid, KLH or HSA, is used, the peptide may be conjugated to the carrier using means standard in the art, including the use of linkers, many of which are commercially available from Pierce Chemical Company, Rockford, IL. Typical linkers include, for example, SMCC and SPDP. Methods of effecting such conjugation are well known.

The foregoing are exemplary of the art-recognized ways to confer immunogenicity on short peptide

sequences such as the Q(K/R)RAA of the invention; any manner of conferring immunogenicity on this sequence may be employed, including synthesis of larger peptides, e.g., 10-100 amino acids, which include this sequence, preferably repetitively.

The vaccines of the invention are administered to persons at risk for rheumatoid arthritis as shown by the haplotypes described above. Thus, individuals with haplotypes corresponding to Dw4, Dw14, Dw15 or DR1 are administered the vaccine.

In one method of RA prevention or amelioration, the immunogenic form of Q(K/R)RAA is administered in conjunction with an immunostimulant and/or immunosuppressant which results in a preferential expansion if T-suppressor and/or cyctotoxic cells at the expense of T-helper cells. Thus, stimulants which expand suppressor/cytotoxic populations but which do not affect helper populations may be used; or substances which actively suppress helper populations, but do not affect suppressor/cytotoxic T-cells may be used, or combinations of these factors may be used. Certain substances which effect this differential expansion are known in the art; however, the invention includes the use of any substance which can be shown to have this effect. Interleukin-6 is an appropriate immunostimulant for suppressor/cytotoxic T-cells. It is often desirable to include IL-2 to enhance this stimulation as well. Cyclosporin A, TGF-beta, and anti-CD4 antibodies (see, e.g., Carteron, N.L., et al., J Immunol (1988) 140:713-716) are appropriate helper cell inhibitors.

By administered "in conjunction with" is meant that either administration is in the same vaccine formulation (containing both the immunogenic form of Q(K/R)RAA and the immunostimulant and/or immunosuppressant) or each of these components may be administered within a conjunctive period of time so that their cooperative effect is

obtained. Suitable conjunctive time periods are of the order of 4 hours to 2 days, preferably 4 hours to 1 day. The immunostimulant/immunosuppressant and the immunogenic peptide may be administered in any order, and protocols of repeated administrations of each or both can also be employed.

Suitable routes of administration are systemic, and include administration by injection such as intravenous, intramuscular, peritoneal, or subcutaneous injection. Subcutaneous or intramuscular administration are preferred. The immunogenic peptide and immunostimulatory/immunosuppressant substance are formulated for injection using standard formulations for the administration of peptides which include suitable excipients such as buffers, Ringer's solution, or Hank's solution, and may further include wetting agents, stabilizers, and the like. Suitable adjuvants may also be included. Other forms of administration, such as transmembrane or transdermal administration using formulations suitable for these routes may also be employed. A wide range of formulations is found, for example, in Remington's Pharmaceutical Sciences, latest edition, Mack Publishing Company, Easton, Pennsylvania.

Suitable amounts of the immunogenic peptide are about 100 ug-100 mg per patient, preferably 10-100 mg. The immunostimulant/immunosuppressant or combination should be administered in an amount according to the potency of the substance chosen; for IL-6, for example, suitable dosage ranges are 1-100 ug/kg, preferably 20-50 ug/kg. It is understood, of course, that dosage level is highly dependent on the particular immunogenic form of the peptide used, the immunostimulant/immunosuppressant selected, the route of administration selected, the nature of the formulation, and the individual response of the subject. Establishment of optimum dosage protocols and regimens for a subject, taking account of the foregoing

parameters, is routinely done and is well within ordinary skill.

In another form of the vaccine, the peptide Q(K/R)RAA or a larger peptide containing this sequence is coupled to an invariant portion of a Class I histocompatibility sequence to generate the active component of the vaccine. The invariant portion of Class I histocompatibility sequences are known and the relevant data are deposited in Genbank and other equivalent data-bases generally known in the art. (See referenced art in Chimini, G., et al., J Exp Med (1989) 169:297-302.)

In addition, the relevant sequences can be found in a publication entitled "Sequences of Proteins of Immunological Interest," 4th ed. (1987), published by the U.S. Dept. of Health and Human Services. On pages 337-358 of this publication are listed the residues occupying positions 1-360 of known Class I MHC antigens from human, murine and other animal sources. A review of these pages shows that the constant regions among the human MHC proteins are positions 1-8; 13-31; 46-53; 84-93; 117-136; 157-176; 200-238; 241-268. In general, peptides from these regions having 10-20 residues, preferably around 12-15 residues, can be conjugated to the relevant Q(K/R)RAA sequence with or without additional extensions derived from those normally surrounding this sequence, as described above.

Methods to conjugate the Class I invariant sequence (which can be synthesized using standard techniques) and the antigen are known in the art, as described above. The conjugation of Class I portion and Q(K/R)RAA peptide can also be effected by recombinant production of the fusion protein. Routes of administration and formulations are also similar to those described above.

The following examples are intended to illustrate, but not to limit, the invention.

Example 1

Homology of the Rheumatoid Arthritis Determinant and Epstein-Barr Virus gp110

As described by Roudier, J., et al., Scand J Immunol (1988) 27:367-371, and Roudier, J., et al., Proc Natl Acad Sci USA (in press), incorporated herein by reference, the QKRAA/QRRAA sequence of the Class II MHC protein, which was known to be related to the incidence of rheumatoid arthritis, was matched through the National Biological Research Foundation database of protein sequences and the Genbank database of DNA sequences to segment 807-816 of the Epstein-Barr virus glycoprotein gp110, which contains the 6-amino acid stretch EQKRAA, matching the HLA Dw4 sequence, followed by a nearly identical second copy, QRAA. Computer analysis of the secondary structure of residues 760-860 of gp110 by the method of Chou and Fassman (Adv Enthymol (1978) 47:45-148) predicts that the relevant QKRAAQRAA stretch is part of an alpha-helix between two hydrophobic regions, representing a structure of a type postulated to constitute the epitope responsible for binding to T-cells. The result of this homology is the ability of this subsequence in Epstein-Barr virus glycoprotein to stimulate the proliferation of T-cells which mediate rheumatoid arthritis and which T-cells recognize the synovial protein antigen conjugated to the Class II MHC protein of susceptible haplotypes Dw4/Dw14/Dw15/DR1.

The enhanced susceptibility of EBV-infected individuals to RA is known; however, this nexus has not been understood. The ability of Epstein-Barr virus (EBV) infection to stimulate the T-cell population to respond to

both the gp110 peptide and the relevant Dw4 peptide is demonstrated in the following examples.

Example 2

Preparation of Peptides

Four peptides representing the relevant portions of the Class II MHC protein haplotypes associated with RA, the gp110 peptide, and a control Dw10 peptide were synthesized with the amino acid sequences:

Dw4:	KDLLEQKRAAVDTYC;
Dw4':	EQKRAAEQKRAA;
Dw14/Dw15/DR1:	KDLLEQRRAAVDTYC;
gp110:	QENQEQKRAAQRAAGC;
Dw10:	KDILEDERAAVDTYC.

These peptides were used as test stimulating peptides for T-cell proliferation or were conjugated to KLH using m-maleimidobenzoyl-n-hydroxysuccinimide as described by Green, N., Cell (1982) 28:477-487; Liu, F.T., et al., Biochemistry (1979) 18:690-697, for use in immunization.

Example 3

Sensitization of T-Cells to Q(K/R)RAA Stimulation by Prior Exposure to EBV

The peptides synthesized in Example 2 were used to determine the response of T-cells in vitro as follows: The method was a modification of that disclosed in Ford, D., Cell Immunol (1983) 79:334-344; Thorley-Lawson, D.A., et al., Proc Natl Acad Sci USA (1987) 84:5384-5388, both incorporated herein by reference. Briefly, peripheral blood mononuclear cells were obtained both from a donor with no history of EBV infection, as indicated by the absence of antibody to the viral capsid antigen (VCA) and

from an otherwise normal subject with a high titer of anti-VCA antibodies, indicating prior exposure to EBV. The mononuclear cells were isolated by isopycnic centrifugation through Ficoll-Hypaque, and were washed three times in RPMI-1640 medium. The cells were then suspended at a density of 10^6 cells/ml in the same medium supplemented with 10% pooled human AB serum, 1% L-glutamine, 100 U/ml penicillin, and 100 ug/ml streptomycin in the presence of 1 ug/ml of a stimulatory peptide selected from the list above. After 1 week culture at 37^oC, a sample of 10^5 cells were taken from the bulk culture to evaluate the primary proliferative response.

In a secondary culturing, the remaining cells were counted and serially diluted in fresh medium supplemented with the same peptide to be tested, or without said peptide as a control. The cells were then distributed in 100 ul aliquots containing 50,000, 25,000, 10,000, or 5,000 viable cells from the primary culture in 96-well round-bottom culture plates and the number of cells in each well adjusted to 10^5 by the addition of irradiated (3,000 rad) peripheral blood mononuclear cells from the same donor. For each cell density, 18 wells with peptide and 6 control wells without peptide were assayed.

After 4 days of this secondary culture, 1 uCi of tritiated thymidine was added to each well 18 hours before cell harvesting on glass fiber filters. For each cell dilution, positive wells were defined as having cpm higher than (mean + 2 standard deviations) of the cpm obtained for 6 control wells. Frequency of precursor T-cells was evaluated by plotting the percentage of negative wells for each dilution against the number of cells per well, as described by Ford et al. (supra).

The results are shown in Figure 1. Panels a and c show the response (in cpm/well) at various dilutions of cells derived from a VCA-positive donor; panels b and d, from VCA-negative donors. Panels a and b were treated

with the gp110 peptide; panels c and d, with the Dw4 peptide.

The results in Figure 1 show that for T-cells derived from VCA-positive donors, either the gp110 peptide or Dw4 peptide was able to stimulate proliferation; for those derived from a VCA-negative donor, no stimulation occurred at any dilution. Corresponding results on a the primary stimulated cultures are shown below in Table 1.

Table 1

	VCA+	VCA-
No stimulation	3976±1025	2691±141
gp110	8720±1227	1976±162
Dw4	9604±1287	2448±578

The foregoing results clearly show that prior exposure to EBV sensitizes a population of T-cells to expansion upon subsequent contact with either the relevant portion of the gp110 peptide or the relevant portion of the Dw4 peptide.

These results further show that the precursor T-cell concentration in an individual with prior exposure to EBV was about 1 in 70,000 T-cells with respect both to gp110 and Dw4.

Example 4

Cross-Reactivity of gp110 and RA-Susceptible Haplotype Antibodies

The peptides prepared in Example 2 and conjugated to KLH were used to raise antibodies in rabbits. New Zealand white rabbits were injected subcutaneously with 1 mg of the conjugate emulsified in complete Freund's adjuvant, and then boosted three times at 3 week

intervals with 1 mg of conjugate in incomplete Freund's adjuvant. The rabbits were bled four days after the last injection and sera were stored at -20°C.

Antibody titers of the resulting sera were tested using an ELISA assay or by immunoblot. The ELISA assay was by the procedure of Luka, J., et al., J Immunol Methods (1984) 67:145-156, incorporated herein by reference. Briefly, the antigen peptide was used to coat ELISA plates, and various solutions of preimmune or immune rabbit sera, diluted in isotonic borate-buffered saline (BBS) were added in 100-ul aliquots to the wells and incubated overnight at 4°C. After washing with BBS, 0.2% Tween-20, bound antibody was detected using alkaline phosphate-conjugated goat antirabbit IgG (Tago, Burlingame, CA). The results are tabulated as absorbance ratios corresponding to O.D. at 405 nm for the immune serum divided by that of the preimmune serum. The antibody titer for each serum is defined as the highest dilution yielding an absorbance ratio of at least 2. In inhibition experiments, candidate inhibitor peptides were added to the antisera and allowed to react overnight at 4°C prior to performance of the ELISA.

ELISA determinations were made when reactivity of antibodies was tested to EBV gp110. The gp110 was purified by affinity chromatography from P3HR1 lymphoblasts according to the method of Kishishita, M., et al., Virology (1984) 133:393-395.

Using this assay, it was demonstrated that a rabbit immunized with the gp110 peptide produced antibodies which recognized both the gp110 peptide and the gp110 protein isolated as described. These results are shown in Figure 2.

Apparently, the epitope recognized on the gp110 protein was that of the peptide, since a 20-fold dilution of serum preincubated with 100 ug/ml of gp110 peptide was no longer capable of binding gp110 protein. These results

indicate that the relevant sequence is an epitope associated with B-cell response to the protein.

Reaction of antisera with Class II MHC proteins was tested by immunoblotting against membrane protein extract from Molt 4, T-cells (control) or from lymphoblastoid cells established from HLA Dw4 or HLA Dw2 homozygous donors (GM03161 and GM066821A, respectively, obtained from Human Genetic Mutant Cell Repository, Camden, NJ). The proteins were extracted with Triton-X114, according to the method of Bordier, C., J Biol Chem (1981) 256:1604-1607.

A membrane extract from 2 x 10^6 cells was loaded into each lane of a 10% polyacrylamide gel containing 0.1% sodium dodecylsulfate and 1 mM 2-mercaptoethanol. After electrophoresis, the reduced and denatured membrane polypeptides were electrophoretically transferred to nitrocellulose sheets, as described by Towbin, H., et al., Proc Natl Acad Sci USA (1979) 76:4350-4354; Billings, P.B., et al., ibid (1983) 80:7104-7108. The filters were then preincubated for one hour in a solution containing 0.05 M borate, 0.15 M NaCl, pH 3, and 3% powdered milk, followed by overnight incubation at 4°C with the antisera diluted at the same dilution. (For inhibition determination, candidate peptides were added to the antibody solutions prior to their incubation with the nitrocellulose sheets.) After extensive washing of the sheets with borate buffer, bound antibody was detected using ^{125}I-protein A (1 mCi/ml, ICN, Irvine, CA). The sheets were incubated for 1 hour with the detecting reagent, washed with BBS, dried, and exposed to Kodak XAR film overnight at -70°C using an intensifier screen.

The ability of Dw4 peptide to raise specific antibodies was also shown by assay of the rabbit antiserum by immunoblot as described above. The results of this assay are shown in Figure 3. The immunoblot in panel A is a control testing anti-DR-beta monoclonal antibody diluted

1:1000 with extracts from Molt 4 (M4) cells, HLA Dw4 homozygous lymphoblastoid cells (D4), or HLA Dw2 homozygous lymphoblastoid cells (D2). Both DR beta proteins react, M4 does not.

In panel B, the gels were incubated with antisera from a rabbit immunized with the Dw4 peptide.

Lane set 1 represents the results when the electrophoresis was run using the membrane extracts alone. Only the D4 extract is immunoreactive with the antibody, confirming the specificity of the antiserum.

Lane set 2 shows the results when the antiserum was preincubated with 10 ug/ml Dw4 peptide; the binding to the membrane-derived D4 peptide in the gel is completely eliminated.

Lane set 3 shows the results when the antiserum was preincubated with the 100 ug/ml of the control peptide Dw10. No effect on the binding of the antibody to the D4 extract was noted.

Lane set 4 shows that preincubation with the related peptide Dw14 at 10 ug/ml was partially effective in eliminating binding to D4; the binding was almost eliminated when the concentration of Dw14 was increased to 100 ug/ml (lane set 5).

A comparison of the sequences of Dw4, Dw2, Dw14, and Dw10 (Figure 4) shows that the presence of residues 67, 70, and 71 are critical for recognition of the Dw4 antibody. The serum from this same rabbit, immunized with Dw4, was shown by ELISA to recognize Dw4 peptide, gp110 peptide, and gp110 protein, with the titers shown in Table 2.

Table 2

Titers of Anti-Dw4 Serum

	Titer
Dw4	10^4
gp110 peptide	10^3
gp110 protein	10^3

The recognition of gp110 protein was inhibited by preincubation of a 100-fold dilution of the serum with either 100 ug/ml gp110 peptide or 100 ug/ml Dw4 peptide. The recognition of gp110 peptide was inhibited by preincubation with 10 ug/ml of either Dw4 or Dw4' peptide (Example 2).

The foregoing results show that the EQKRAA sequence shared by gp110 and HLA Dw4 is the basis for serological cross-reaction.

Example 5

Conjugation to Class I Constant Region

The following peptides, representing constant regions of the human Class I MHC glycoprotein are constructed:

A-Y-D-G-K-D-Y-I-A-L-(K/N)-E-D-L-(R/S)-S-W-T-A-A-(D/N)-(M/T)-A-A-Q

representing positions 117-141 of the human HLA Class I protein; and

E-A-T-L-R-C-W-A-L-(G/S)-F-Y-P-A-E-I-T-L-T-W-Q-R-D-G-E-D-Q-T-Q-D-T-E-L-V-E-T-R-P-A-G-D-G-T-F

representing positions 198-241.

Fragments of these peptides containing 12-15 residues from the sequences shown are also prepared.

These peptides are conjugated using dicyclohexylcarbodiimide to the peptide of the sequence Q(K/R)RAA and formulated into vaccines.

Alternatively, the gene encoding the desired portion of the above peptides with extensions of the residues Q(K/R)RAA (including constructions containing codons for multimers of the pentapeptide) are constructed and inserted into standard recombinant expression systems for production of fusion proteins. The resulting fusions are then formulated into vaccines as described above.

Claims

1. A method to immunize a subject against rheumatoid arthritis, which method comprises administering to said subject in need of such immunization,

an amount of a peptide comprising the sequence Q(K/R)RAA in immunogenic form effective to stimulate the T-immune system,

in conjunction with at least one immunostimulant and/or immunosuppressant capable of effecting the preferential expansion of suppressor and/or cytotoxic T-cells in comparison to helper T-cells.

2. The method of claim 1 wherein the immunostimulant is interleukin-6 (IL-6), with or without IL-2.

3. The method of claim 1 wherein the immunosuppressant is selected from cyclosporin A, TGF-beta, and antibodies immunoreactive with CD4 surface antigen.

4. A pharmaceutical composition which comprises, in admixture, a peptide containing the sequence Q(K/R)RAA in immunogenic form and an immunostimulant and/or immunosuppressant capable of effecting the preferential expansion of suppressor and/or cytotoxic T-cells as compared to helper T-cells.

5. The composition of claim 4 wherein the immunostimulant is interleukin-6 (IL-6), with or without IL-2.

6. The composition of claim 4 wherein the immunosuppressant is selected from cyclosporin A, TGF-

Fragments of these peptides containing 12-15 residues from the sequences shown are also prepared.

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Alternatively, the gene encoding the desired portion of the above peptides with extensions of the residues Q(K/R)RAA (including constructions containing codons for multimers of the pentapeptide) are constructed and inserted into standard recombinant expression systems for production of fusion proteins. The resulting fusions are then formulated into vaccines as described above.

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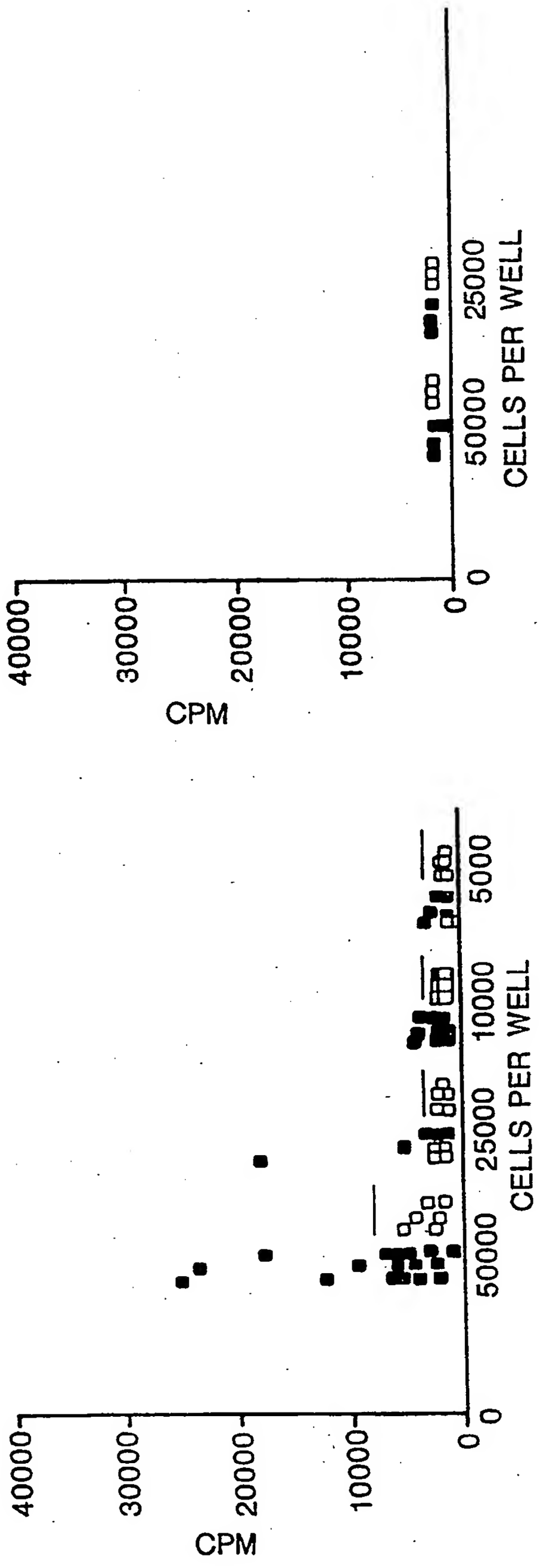
6. The composition of claim 4 wherein the immunosuppressant is selected from cyclosporin A, TGF-

beta, and antibodies immunoreactive with CD4 surface antigen.

7. A method to immunize a subject against rheumatoid arthritis which method comprises administering to said subject in need of such immunization an amount of a peptide comprising the sequence Q(K/R)RAA conjugated to the invariant portion of the Class I histocompatibility sequence effective to stimulate the production of specific suppressor and/or cytotoxic T-cells.

8. A vaccine useful in immunizing human subjects against rheumatoid arthritis which contains as active ingredient a peptide comprising the amino acid sequence Q(K/R)RAA conjugated to the invariant portion of a Class I histocompatibility sequence.

9. A composition of matter which consists essentially of a peptide comprising the sequence Q(K/R)RAA conjugated to the invariant portion of a Class I histocompatibility sequence.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CELLS PER WELL

FIG. 1B

40000
30000
20000
10000
0
CPM
0
50000
25000
10000
5000
CELLS PER WELL

FIG. 1A

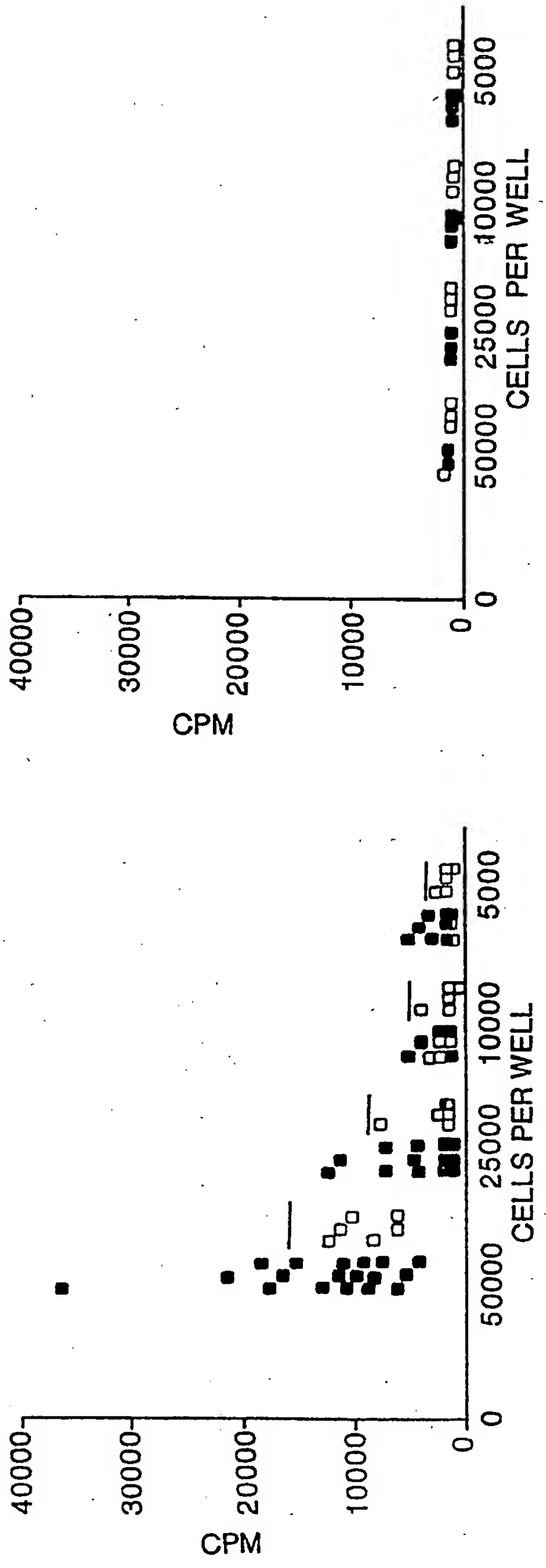
40000
30000
20000
10000
0
CPM
0
50000
25000
10000
5000
CELLS PER WELL

FIG. ID

FIG. IC

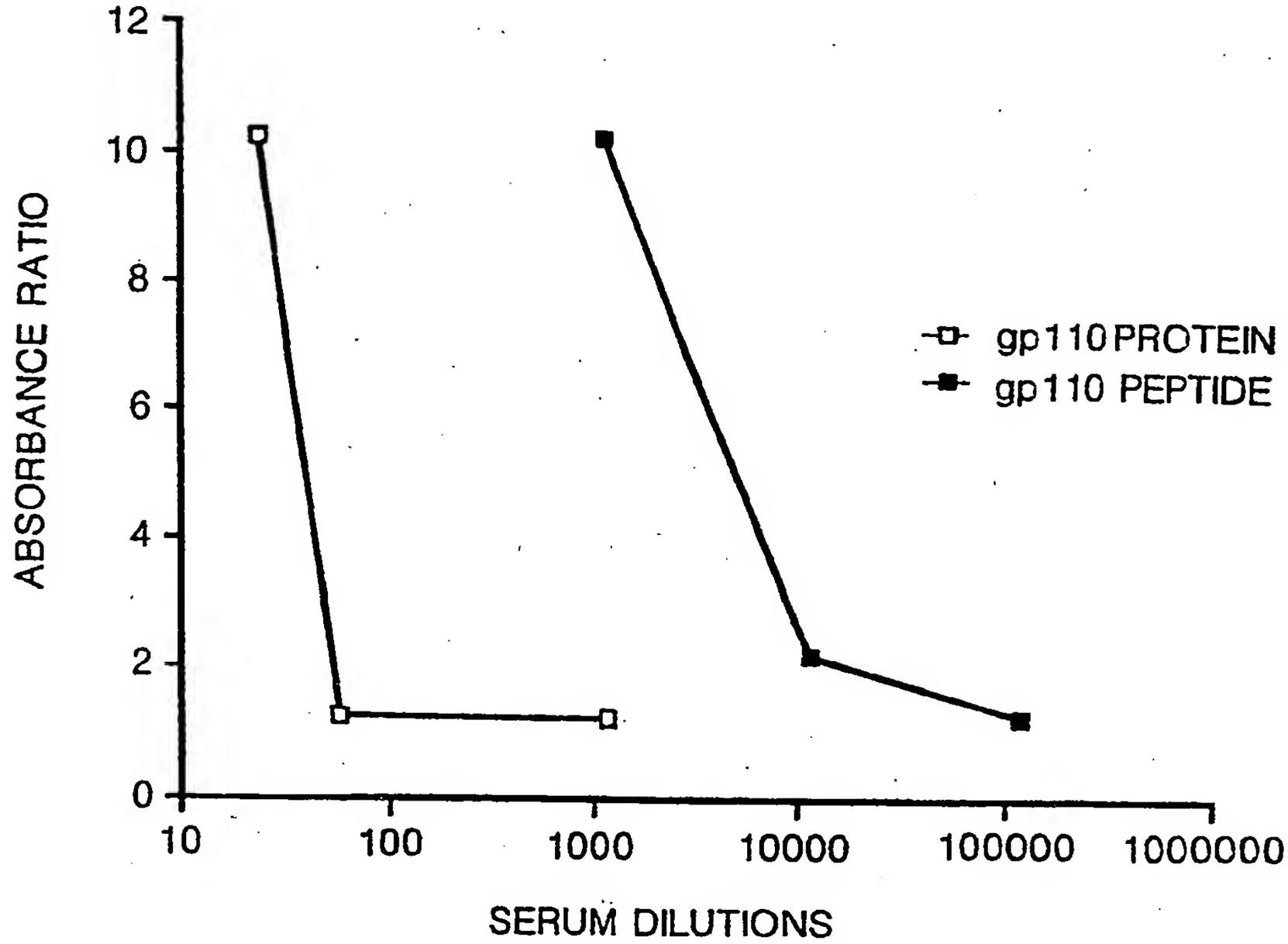
12
10
8
6
4
2
0
ABSORBANCE RATIO
gp110 PROTEIN
gp110 PEPTIDE
10
100
1000
10000
100000
1000000
SERUM DILUTIONS

FIG. 2

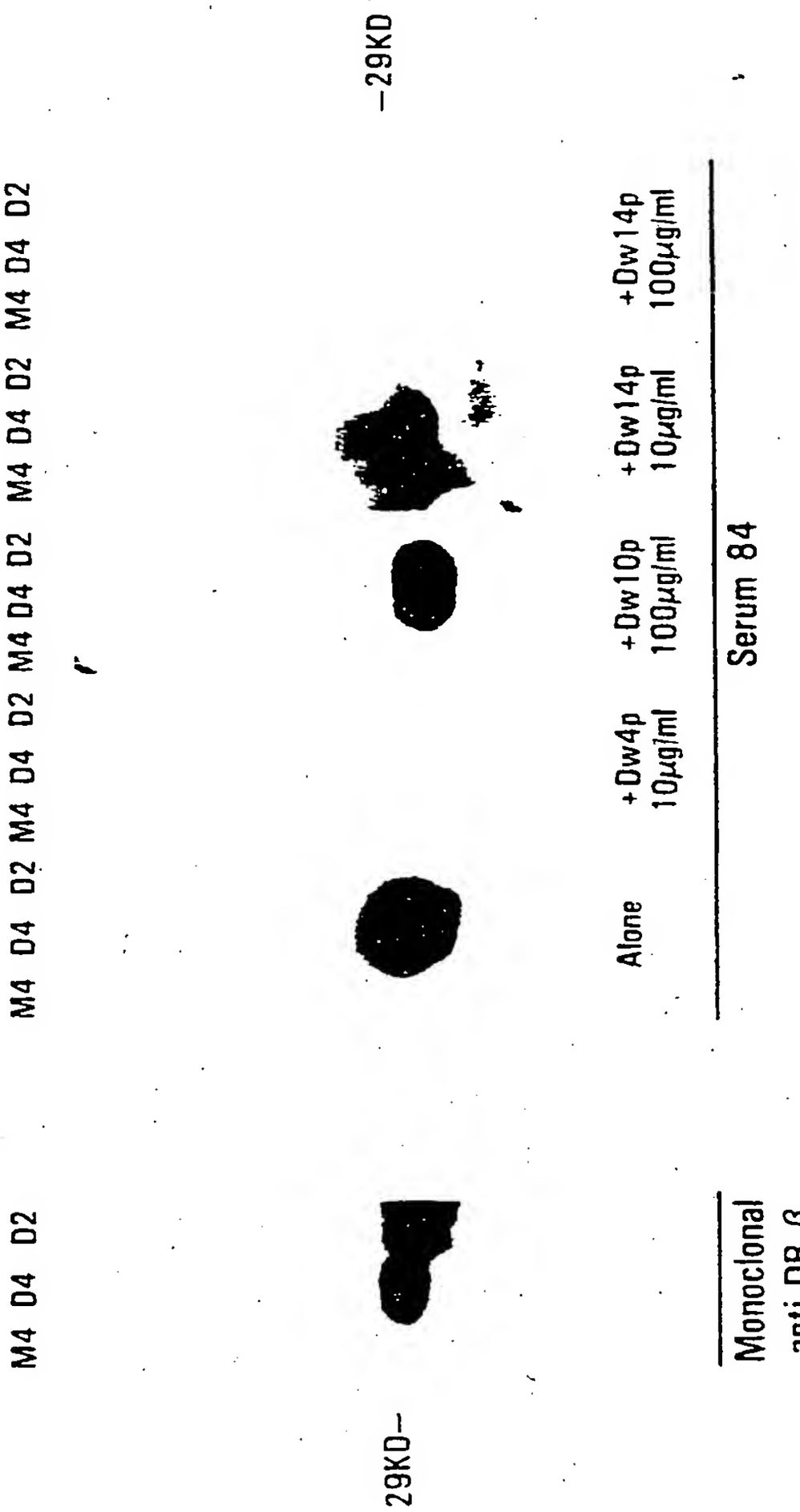
M4 D4 D2 M4 D4 D2 M4 D4 D2 M4 D4 D2 M4 D4 D2
29KD
Alone
+Dw4p 10µg/ml
+Dw10p 100µg/ml
+Dw14p 10µg/ml
Serum 84
+Dw14p 100µg/ml
Monoclonal anti DR β
29KD

FIG. 3A

FIG. 3B

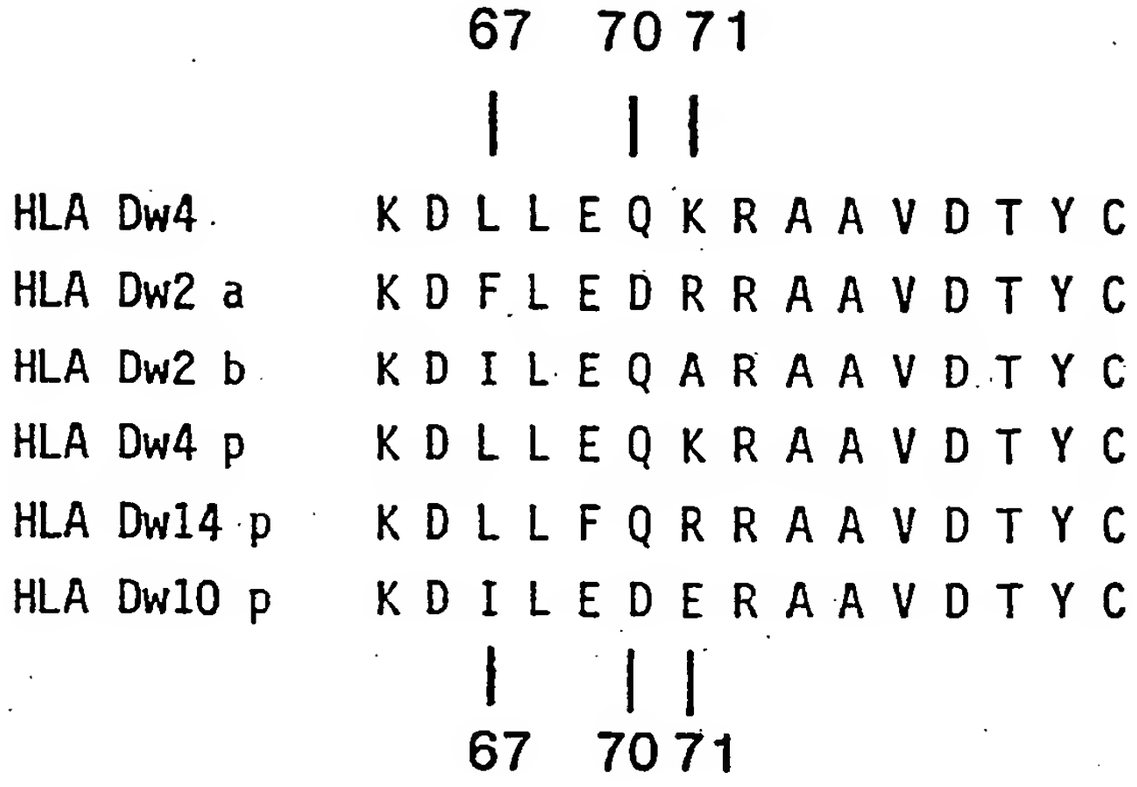
67 70 71
HLA Dw4 K D L L E Q K R A A V D T Y C
HLA Dw2 a K D F L E D R R A A V D T Y C
HLA Dw2 b K D I L E Q A R A A V D T Y C
HLA Dw4 p K D L L E Q K R A A V D T Y C
HLA Dw14 p K D L L F Q R R A A V D T Y C
HLA Dw10 p K D I L E D E R A A V D T Y C
67 70 71

FIG. 4

INTERNATIONAL SEARCH REPORT

International Application No PCT/US90/03038

I. CLASSIFICATION OF SUBJECT MATTER (If several classification symbols apply, indicate all) [3]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC(5): A61K 37/02,37/66,39/395,39/39,39/00

U.S. CL.: 514/2,8,17; 424/85.1,85.2,85.6,85.8,85.91,88

II. FIELDS SEARCHED

Minimum Documentation Searched [4]

Classification System	Classification Symbols
U.S.	514/2,8,17,885,825; 424/85.1,85.2,85.4,85.6, 85.8,85.91,88

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [5]

Computer search of Biosis, Medline and WPI/WPIL for: Q(K/R)R AA or DR1 or DR4 or Class II Ag/protein or T-cell receptor to treat Rheumatoid Arthritis; Q(K/R)RAA conjugated to class I Ag and immunosuppressants/immunostimulant

III. DOCUMENTS CONSIDERED TO BE RELEVANT [14]

Category *	Citation of Document, [16] with Indication, where appropriate, of the relevant passages [17]	Relevant to Claim No. [18]
Y	Journal of Immunology, Vol. 140, No. 2, issued January 1988, "Identification of the DRw10 $DR\beta_1$-Chain Allele As Encoding A Polymorphic Class II Major Histocompatibility Complex Epitope Otherwise Restricted to $DR\beta_2$ Molecules of The DRw53 Type", (MATSUYAMA ET AL.), pages 537-43, See pages 537 and 541-42.	1-9
Y	Journal Clinical Invest., Vol. 77, Issued March 1986, "Shared T Cell Recognition Sites on Human Histocompatibility Leukocyte Antigen Class II Molecules of Patients with Seropositive Rheumatoid Arthritis", (GORONZY ET AL.) pages 1042-49, See pages 1042, 1046-49.	1-9
Y	Clin. Exp. Immunol. Vol. 70, issued 1987, "Synovial Fluid Mononuclear Cells Exhibit a Spontaneous HLA-DR Drive Proliferative Response", (DUKE), Biosis Abstract No. 06436059, See the abstract.	1-6
Y	Journal Immunol., Vol. 141, No. 5, issued 01 September 1988, "IL-6/bsf-2 Functions As a Killer Helper Factor In the In Vitro Induction of Cytotoxic T Cells", (OKADA ET AL.), PAGES 1543-49, See page 1543.	1-2,4-5

* Special categories of cited documents: [15]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search [2]	Date of Mailing of this International Search Report [2]
23 AUGUST 1990	28 SEP 1990
International Searching Authority [1]	**Signature of Authorized Officer**
ISA/US	NGUYEN NGOC-HO For INTERNATIONAL DIVISION GARNETTE D. DRAPER Ngoc-Ho Nguyen

Form PCT/ISA/210 (second sheet) (May 1986)

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED)

Y	Journal Immunol., Vol. 139, No.5, issued 01 September 1987, "Inhibition of The Helper Function of Murine T Cells With Fab'-Anti-L3T4 Ricin A Chain Immunotoxin", (STREET ET AL.), pages 1734-38, See page 1734.	1,3,4,6
Y	Journal Immunol., Vol. 141, issued 15 September 1988, "Effects of Anti-Lyt-2 and Anti-L3T4 Monoclonal Antibodies on The Function of Cytotoxic T Lymphocyte/ Helper T Lymphocyte Hybrid T Cell Clones", (HAVRAN ET AL.), PAGES 1808-12, See page 1808.	1,3,4,6
Y	Chemical Abstract, Vol. 108, issued 1988, The Shared Epitope Hypothesis. An Approach to Understanding the Molecular Genetics of Susceptibility to Rheumatoid Arthritis," (GREGERSEN), Abstract No. 107138b, Arthritis Rheum., 1987, 30(11), 1205-13, See the abstract.	1-9

FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

V. ☐ OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE [1]

This International search report has not been established in respect of certain claims under Article 17(2) (a) for the following reasons:

1. ☐ Claim numbers ....., because they relate to subject matter [1] not required to be searched by this Authority, namely:

2. ☐ Claim numbers ....., because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international sparch can be carried out [1], specifically:

3. ☐ Claim numbers _____, because they are dependent claims not drafted in accordance with the second and third sentences of PCT Rule 6.4(a).

VI. ☒ OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING [2]

This International Searching Authority found multiple inventions in this international application as follows:

Group I, claims 1-2 and 4-5 to composition of Q(K/R)RAA and immunostimulants, and a method of treating RA, classified in 424/85.2 and 514/2; Group II, claims 1,3,4 and 6 to composition of Q(K/R)RAA and immunosuppressants, and method of treating RA, classified in 424/85.8 and 514/2;

(See Attachment)

1. ☒ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims of the international application. SEE Telephone Practice

2. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the international application for which fees were paid, specifically claims:

3. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers:

4. ☐ As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additional fee.

Remark on Protest

☐ The additional search fees were accompanied by applicant's protest.

☐ No protest accompanied the payment of additional search fees.

ATTACHMENT TO FOR PCT/ISA 210, PART VI

CONTINUATION:

Group III, claims 7-9 to a composition and vaccine comprising Q(K/R)RAA conjugated to the invariant portion of the class I histocompatibility sequence, and method for treating RA, classified in 424/85.91 and 424/88.

ATTACHMENT TO CHAPTER I PCT TELEPHONE PRACTICE FOR LACK OF UNITY OF INVENTION

DETAILED REASONS FOR HOLDING LACK OF UNITY OF INVENTION

The invention of Group I, claims 1-2 and 4-5 is drawn to a composition of the peptide Q(K/R)RAA and the immunostimulants IL-2 and/or IL-6, and to the use of the composition to treat Rheumatoid Arthritis (RA), classified in 424/85.2 AND 514/2.

Group II, claims 1,3,4 and 6 is drawn to a composition of the peptide Q(K/R)RAA and the immunosuppressants of TGF, Cyclosporin A, or anti-CD4/anti-L3T4, and to the use of this composition to treat RA, classified in 424/85.8 and 514/2.

Group III, claims 7-9 is drawn to a composition and vaccine of Q(K/R)RAA conjugated to the invariant portion of the class I histocompatibility sequence and to a method of using the conjugate to treat RA, classified in 424/85.91 and 424/88.

PCT Rule 13.2 permits claims to one product, one method of making the product, and to one method of using the product. The first appearing invention of Group I represents such a combination as set forth by rule 13.2. However, the inventions of Groups II and III represent multiple products (compositions) and their use. These compositions are functionally distinct and encompass various species that are used as either immunostimulants or immuno suppressants, and these groups are not so linked as to form a single general inventive concept. There are no provisions in PCT Rule 13.2 for claims covering multiple products (compositions) and their uses.